THE MORPHOLOGY OF *RHIZOCTONIA SOLANI* KÜHN AND METHODS FOR INDUCING THE FORMATION OF ITS PERFECT STAGE[1]

by

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Introduction

Rhizoctonia solani Kühn is one of the most widely distributed pathogenic fungi naturally inhabiting soil (26). Its many recognizable strains show that it is a complex species. The increasing number of publications dealing with diseases caused by this fungus in recent years and its extremely wide host range testify to its economic importance.

The infrequency of basidial production by *Rhizoctonia* spp. has augmented the taxonomic confusion and delayed genetic studies of this group (60). Parmeter (41) states that precise identification of mycelia is difficult. Because the mycelial system is basically simple and provides only limited diagnostic features, the range of variation within a species is often great. This appears to be especially true of many basidiomycetes with brown, relatively large hyphae (26).

Most of the papers on *R. solani* have not included data on its perfect stage, due to a rather infrequency of the production of *R. solani* both *in vivo* and *in vitro*. It seems that the factors influencing the formation of the perfect stage of *R. solani* are still not completely known.

This paper attempts to review the literatures on the morphology and the methods for inducing formation of the perfect stage of *R. solani*.

General Characteristics of the Imperfect Stage of R. solani

The first description of *R. solani* causing plant disease was given by Duhamel in 1728 (17). But the genus "*Rhizoctonia*" was first established by De Candolle in 1815 to include the species *Rhizoctonia crocorum* (Pers.) DC. (53). After De Candolle established the genus, a large number of species were described in the 19th century. *R. solani* was first reported by Kühn on potato in 1858 (32).

The first valid description of the genus "*Rhizoctonia*" is that given by Fries in his Systema Mycologicum (24), which is the starting point for mycological

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nomenclature. According to this description, the genus is characterized by the absence of any kind of fruiting bodies and the presence of sclerotia, varying in shape and size, growing together, cartilagenous to fleshy, and of uniform texture within. The cortex of the sclerotia is very thin, membranous, and persistent. Root-like cottony threads growing out in fascicles from the corners of the sclerotia join several sclerotia together. The diagnosis specifies roots of plant as the habitat of these fungi.

In 1915, Dugger made a classical study on the genus *Rhizoctonia* (14). He recognized only two main species, *R. crocorum* and *R. solani*. These two representative species were shown to possess a vegetative mycelium characterized by 1) constriction of the hyphal branches at their point of origin, 2) presence of a septum close to the point of branching, and 3) occurrence of lateral growth or branching, often at right or acute angles.

The monilioid cells produced by mycelium are also one of the characters of these fungi (16, 53). Dugger (14) distingushes *R. solani* from *R. crocorum* by the difference of monilioid cells. Ruhland (49) takes special note of the monilioid cells of a *Rhizoctonia*-like fungus from Germany and erects a new genus *Moniliopsis* to include his fungus under the name *M. aderholdi*. *M. aderholdi* is later considered identical with *R. solani* (15, 57), and it is proposed that the genus *Moniliopsis* be merged with *Rhizoctonia* (*Moniliopsis* has since come to be treated as a genus *Ambiguum*); monilioid cell is also termed "chlamydospore" (52). The characters of chlamydospore of *Rhizoctonia* are very consistent within a species (52). It is considered to have a great advantage in separating species of *Rhizoctonia* (42, 53).

R. solani grows well on culture media (64). Potato-dextrose agar and Richards's medium give the most rapid growth of this fungus (1). On potato-dextrose agar, the colony is brown with flaty and cottony aerial hyphae (53). The vegetative hyphae are septate, hyaline at first, later becoming brown, occurring in visible brown strands on the host substrate (64). Branches are inclined in the direction of growth when young. They assume a right angle in relation to the main hyphae, and constrict at the point of the branching when old (47, 53, 64). The first septum is a few microns beyond the point of original (53). The main hypha The hyphae are devoid of clamp connections (14,16,61). The width of main hyphae varies in different isolates. Saksena and Vaartaja (53) reported that the width of main hyphae of 3 isolates measured 7-11 μ, 4.5-8 μ, and 4.5-6 μ respectively. The average diameter of main hyphae was 5.13-8.0 (–14) μ in the isolate measured by the author (61).

The most mature hyphae are very dark, rather rigid, the cell walls thicker, with some cruciform cells (also called monilioid cells or chlamydospores), eventually developing into brown sclerotia (64).

Sclerotia are formed abundantly in 7-day-old colonies on the surface of potato-dextrose agar (61). The sclerotia are brown, globose, more or less flattened, and

smooth on the surface (44, 61).

The morphological characters, such as width of mycelium, and location, size and shape of sclerotia have been used for separating *Rhizoctonia* species (34). Since the asexual structures vary from isolate to isolate within the same species (53), Kernkamp *et al.* (28) point out that these structures alone are of little taxonomic value.

The optimum temperature for mycelial growth of *R. solani* on culture media is from 25 to 30 C, minimum of about 8 C, and maximum of 31 to 35 C (64). Bosch (5) states that the minimum growth temperature is 4 C, optimum 26 C. Ashour *et al.* (1) report that the optimum temperature on Richards's medium is 30 C. It is evident that difference in temperature optima varies with isolate and culture media concerned (64).

The pH value of culture medium also has great influence on the growth of mycelium. In general, the best growth occurs between pH 4.2 and 6 (1). Bosch (5) reports that optimum pH for growth is found to lie between 5.5 and 7, no growth at 3, but upward of 3.6 they develop well up to beyond 7. Blair (3) states that the mycelium is able to grow on culture media under pH 3.0 to 8.6. The best growth of mycelium in the soil lies between pH 5.8 and 8.1. Schaffnit *et al.* (55) mention that changing soil pH value from 4.3 or 4.8 to 7.06 or 6.26 can stimulate the growth of *R. solani* in soil.

The best growth of *R. solani* occurs at the relative humidity of 92.9—100% (1). Schneider (56) reports that *R. solani* suffers most from a reduction in humidity, the minimum value permiting mycelial growth is 99.5%. Soil moisture is regarded by most workers as secondary to soil temperature in influence on the growth of *R. solani* in the soil and causing disease on plants (64).

The original concept that *Rhizoctonia* inhabits only soil and plant roots has also been modified (64). Four phases of disease, such as damping-off, stem canker and root rot, storage organ decay, and folliage blight or spot are reported by many investigators (1, 16, 25, 28, 30, 53, 64). *R. solani* can change the phase of the disease when the environment changes. Pinckard and Luke (45) report that *R. solani* which is original agent causing damping-off of cotton seedling is now a primary cause of cotton ball rot in Louisiana.

General Characteristics of the perfect Stage of R. solani

The perfect stage of *R. solani* was not discovered until early 20th century. Patouillard (43) in France first described the basidial stage as *Hypochnus filamentosa* in 1891, and Prillieux and Delacroix (46) described it in the same year and same country, on potato stem as *H. solani*. However, none of the investigators suspected a connection with *R. solani* at that time.

In 1903, Rolfs (48), working with the *Rhizoctonia* disease of potato in Colorado,

found a basidiomycete constantly associated with this fungus. He germinated basidiospores and secured a fungus identical with *R. solani*. Rolfs sent his material to Burt, who identified it as being close to *Corticium vagum*, a saprophyte on pine bark described by Berkeley in 1873 (20). Burt classified it as a variety of this species and named it *C. vagum* Berk, & Curt. var. *solani* Burt, because of differences in the number of sterigmata per basidium and in the size and shape of the basidiospores. Linder (40), supported by Rogers (47) and Cunningham (8), regarded these differences, together with difference in habitat, as being important enough to separate Rolfs's material as a different species. Rogers (47) accepted Patouillard's species but referred it to be the genus *Pellicularia* which was established by Cooke in 1876 for *P. koleroga*. He named it *Pellicularia filamentosa* (Pat.) Rogers.

According to Rogers (47), *P. filamentosa* is the combination of *Hypochnus filamentosa* Pat. 1891, *H. solani* Prill. and Del. 1891, *C. vagum* var *solani* Burt ex Rolfs 1903, *C. solani* (Prill. and Del.) Bourd and Galz. 1928, *Botryobasidium solani* (Prill. and Del.) Donk, *C. areolatum* Stahel. 1940, nec *C. areolatum* Bres. 1925, *Oidium citri* Bondar. 1929 (*nomen nudum*), and *C. microsclerotia* Weber 1939 (*nomen nudum*). The fungus is characterized by subcylindrical, barrel-shaped, or obpiriform, clavate basidia, formed on the subbasidial hyphae in small, imperfectly symmetrical cymes, 10 or 12 to 18 or 23 by 8 to 11 or 12.5 μ, bearing 4 horn-shaped sterigmata, 3 or 5.5 to 20 by 1.5 to 3.5 or 4.5 μ, and ellipsoid or oblong-ellipsoid, truncate to spiculate spores, 7 to 12.5 by 4 to 7 μ, occasionally germinating by means of a stout promycelium carrying a similar secondary spore.

In 1954 Donk (9) reported that *Pellicularia* was *nomen confusum*, being based on the vegetative hyphae of a resupinate basidiomycete and the associated spores of one of the Fungi Imperfecti. He considered that *Botryobasidium* Donk (1931) was the correct generic name.

After a series of studies, Donk (10, 11, 12, 13) recombined *H. cucumeris*, *H. filamentosa*, *R. solani*, *P. filamentosa*, and *Ceratobasidium filamentosa* to *Thanatephorus cucumeris* (Frank) Donk 1956.

In 1962, Warcup and Talbot (65) agreed with Donk, and reported that *Pellicularia* was a *nomen confusum* as donk mentioned (9), and claimed that there was ample justification for accepting the generic segregates from *Corticium* as Donk suggested in 1958 (12).

According to Warcup and Talbot (65). *T. cucumeris* is characterized by fructification resupinate, delicate, thin. white, pruinose to floccose or arachnoid, with a discontinuous hymenium formed of clusters of basidia. Basidia 15-18 x 8-10 μ, in cymose terminal clusters on erect, short-celled hyphae; basidia a barrel shaped or oblong, no median constriction or stipitate base, broadly attached, not much wider than the supporting hypha; sterigmata up to 15-25 μ long and 3 μ wide at the base, 4 per basidium, stout, more or less straight and slightly divergent, not curved like

horns; spores 7-9 x 4-6.3 μ, hyaline, not amyloid, smooth, with walled, oblong to ellipsoid with one side flattened, or broad ovoid, with a truncate apiculus, germinating by repitition.

Since there are great variations in morphology, physiology and pathogenicity among strains, it is commonly believed that strains included under *R. solani* may represent more than one species (20). Vanterpool (63), working with *R. solani* strains isolated from blighted flax seedlings, finds that they have *C. praticola* and not *P. filamentosa* as their perfect stage. In a preliminary studies on the sexual stage of *R. solani*, Hawn and Vanterpool (25) report that the perfect stage of a flax strain of *R. solani* is closely with those of *P. filamentosa* in growth characters and morpholgical dimensions, except that the sterigmata averaged three times long (26 μ). This latter character suggests close relationship to *C. praticola*.

In 1956, Flentje (20) induced 28 isolates of *R. solani* to produce the prefect stage *in vitro*. He found that four of the isolates were identical with *Pellicularia praticola* (=*C. praticola*), and the remainder were isolates of *P. filamentosa*. *P. praticola* is characterized by 1) one to five sterigma per basidium, mostly three, 2) basidia in loosely arranged flat plaques following irregular recemose branching (20).

Strains possessing different nuclear number in the vegetative cells show that they will produce different perfect stage. Parmeter *et al.* (42) report that *R. solani*-like binucleate isolate produce *Ceratobasidium* perfect stage; multinucleate isolates produce *T. cucumeris* perfect stage.

Before the discovery of binucleate strains, all studies of the vegetative cells of *R. solani* indicate that they are multinucleate (25, 26, 51, 54). It seems that *T. cucumeris* is more common than *Ceratobasidium* sp. in the nature.

According to Saksena (51), Boidin (4), Sanford and Skoropad (54), and Flentje *et al.* (23) the number of nuclei per cell of *T. cucumeris* varies from 4-25, 6-30, 2-25, and 4-12. Sanford and Skoropad (54) state that the numbers are commonly 4-8 in the tip cells and 6-11 in the older vegetative hyphae. Hawn and Vanterpool (26) suggest that 8 nuclei are common in the tip cells. Boidin (4) finds that the number of nuclei differs in successive cells of a hypha. According to Boidin (4), there is usually, but not always, an equal number of nuclei in each cell.

Saksena (51) suggests that nuclear division in the vegetative cells is unlike the classic mitotic division of nuclei in higher plants. Flentje *et al.* (23) state that nuclear division in the vegetative cells is found to be conjugate, followed by an even segregation of the daughter nuclei.

Sanford and Skoropad (52) claim to have observed nuclear migration through septal pores and Saksena (49) illustrates structures he considers to be nuclei wedged in septal pores of stained cells. Hawn and Vanterpool (26) and Flentje *et al.* (23) find no evidence of nuclear migration through septa.

The change from vegetative to reproductive phase is associated with septation of

hyphae cutting off cells with only 2 nuclei (23, 26). In the basidia karyogamy and meiosis occur, resulting in 4 haploid nuclei which migrate through 4 sterigma to form 4 uninucleate spores (23). Abberrations also occur in the reproductive phase; 3 nuclei instead of 2 are sometime migrate from the basidium into 1 basidiospore (23, 50).

Hawn and Vanterpool (26) state that there are 12 chromosomes in the diploid nucleus in the basidium and 6 chromosomes in the haploid nucleus in the basidiospore.

The heaviest spore production and discharge occur at night (7, 45), sporulation in the daytime is low or absent (7). Basidiospores are discharged by the drop excretion mechanism (26). Basidiospores germinate in 10 to 12 hr. after discharge and cause infection when present on susceptible plants under suitable environmental conditions (45). Single-basidiospore cultures differ among themselves and from parent strains in morphology, culture characters, growth rates, and pathogenicity (19, 21, 26, 39).

Table 1. The measurement (μ) of basidial structures of *Rhizoctonia solani* Kühn (=*Thanatephorus cucumeris* (Frank) Donk)

Measured by	Year	Basidia	Sterigmata	Spores
Rogers	1943	(10-)12-18(-23) x 8-11(-12.5)	(3-)5.5-12(-20) x 1.5-3.5(-4.5)	7.0-12.5 x 4-7
Warcup & Talbot	1962	15-18 x 8-10	15(-25) x 3	7-9 x 4-6.3
Tu	1967	14-19(-22) x 7.6-10(-11)	4.3-14 x 1.5-3.7	7.1-10 x 3.9-6.8

Methods for Inducing the Formation of the Perfect Stage of *R. solani*:

There are many techniques commonly used for inducing formation of the sexual stage of *R. solani* in the laboratory. These methods can be concluded into 4 groups. They are 1) fructificationon the surface of agar, 2) fructification on plant tissues, 3) fructification on the soil surface, and 4) the others.

1. Fructification on the Surface of Agar:

Since the early years, this method has been widely used for inducing some isolates of *R. solani* to fruit in the laboratory. However, great difficulty also has been experienced in obtaining and controlling the formation of the sexual stage of *R. solani* in artificial culture (26). Muller (29), Ullstrup (62), Exner and Chiton (19), Kotila (30), Flentje (20), Hawn and Vanterpool (26), Whitney and Parmeter (67), and Papavizas (39) have met with varying degrees ot success in obtaining the perfect stage of various strains of *R. solani* in culture by applying the principle of growing the fungus on a rich medium (growth media) and then transferring it to a weaker one or water agar (sporulation media).

Kotila (29) finds that the formation of the sexual stage of *T. praticolus* (*C. praticola*, *P. praticola*), a fungus very closely relates to *T. cucumeris*, can be fairly well controlled on artificial media. Stretton *et al.* (60) support Kotila's result on *T. praticolus*. For *T. cucumeris* (=*R. solani*), Stretton *et al.* (60) point out that nonpathogenic isolates can fruit on agar or soil, but pathogenic isolates only on soil.

According to Hawn and Vanterpool (26), the usual method of growing the fungus on rich nutrient media and then transferring it to water agar is successfully used in his experiment to induce the hymenial development. Transfers are made from approximately 30-day-old malt agar or potato-dextrose agar test tube cultures to Petri plates containing thin layer of 2% water agar. The inoculated plates are placed under a bell jar in diffuse light in front of a north window. A temperature range of 19 to 25 C and 70-80% relative humidity give the best results.

After growth a period on rich agar, some isolates produce the sexual stage on the soil-extract agar instead of water agar (37). Flentje (20) reports that the most satisfactory method is to incubate the isolates on potato-marmite-dextrose agar at 25 C for 7-8 days and then transfer them to soil-extract agar, after a further 7 days of incubation at 25 C, the Petri dishes are removed from the incubator to a laboratory bench. The soil-extract agar is made up by autoclaving 1 kg. air-dried soil with 1 liter distilled water for 30 min. at 1 atm. After standing over night, the supernatant liquid is filtered through filter paper pulp. To this 1.0 g. dextrose, 0.1 g. yeast extract, 0.2 g. KH_2PO_4 and 20 g. agar are added then the liquid is made up to 1 liter.

2. Fructification on Plant Tissues:

Some strains of *R. solani* fruit on plant tissues. Sims (59) points out that basidiospores of *R. solani* develop on plants of cotton, alligator weed, wandering jew, potato, or sugar beet after the plants have been inoculated with mycelial suspensions of certain isolates and maintained under bell jar at 20 C. He also reports that *R. solani* develops basidiospores on hyphae arising from 2 campadible hyphae that have developed on potato broth amended with sucrose or maltose 10 days after transfer of fhe compatible hyphae to alligator stems and roots.

Flentje (20) mentions that *R. solani* fruits on the stems of plants (potato and tomato) growing in inoculated soil. The fructification occurs 28-35 days after inoculation. The hymenium develops as tenucus, mealy to powdery, whitish-gray layer adhering closely to the plant stem and extending from soil level to 4 or 5 cm above this. The fungus is entirely external but one strain penetrats into the epidermal cells of potato and tomato stems, producing a browning of the stem.

Some strains of *R. solani* produce the perfect stage on diseased plants. Pinckard and Luke (45) state that *R. solani* causes cotton boll rot and produces the perfect stage on cotton near the soil surface. Some strains produce the sexual stage on the less susceptible plant rather than the most susceptible plant. According to Neal (35), cotton-leaf-*Rhizoctonia* can not fruit on cotton, but Kotila (30) observed it

producing perfect stage on artificially inoculated spots of sugar beets.

3. Fructification on the Soil Surface:

The fact that a large percentage of *R. solani* fruit on Waite Institute soil suggests that the method for obtaining basidial stage of *R. solani* from the soil surface can be successfully used in clarifying the present confusion in the taxonomy of the genus *Rhizoctonia* (20, 60). In 1956, Flentje (20) induced *R. solani* to produce the perfect stage on the surface of inoculated Waite Institute soil. He reported that the hymenium appeared from 5 to 21 days after the soil inoculated with the corn-meal inoculum. The hymenium consisted of a tenuous, mealy to powdery, white grey layer. The greatest intensity of basidia and basidiospores occurred at 5 to 10 days after they first appeared and lasted a further 5-7 days. Towards the end of this time they gradually turned brown and disappeared.

In 1964, Stretton *et al.* (60) developed two simple methods for inducing sexual fruiting of *R. solani* on soil. The first method was th so-called "soil-plate method". Isolates were grown on potato-marmite (commercial yeast-vegetable extract)-dextrose agar (PMDA) in 9-cm Petri dishes for 7-10 days. The lids were then removed, each culture was watered 1-3 times a day to keep it moist. Fruiting usually occurred after 3-14 days. The second was the so-called "inoculated soil method". Isolates were grown on PMDA for 7-10 days, then each culture was either macerated with scalpel or ground with sterile sand in a mortar, and incorporated into soil (approximately 30 cm^2 culture/300 g. soil). Inoculated soils were placed in waxed or plastic-coated paper drinking cups and watered 1-3 times a day to keep the soil moist.

In a more recent report, Parmeter *et al.* (40) successfully induced *Rhizoctonia* spp. to produce the perfect stage on the soil surface by the modified soil-plate method. Isolates were grown for a few days on autoclaved bean pods in Petri dishes. Resulting colonies were then covered with a 5-10 mm layer of autoclaved soil, and the dishes were incubated without lids at room temperature (18-22 C). The soil was watered periodically to keep it moist. Small pieces (3-5 mm^2) of cellophane were scattered on the soil surface. When fruiting occurred, usually within 1-2 weeks, pieces of cellophane bearing fruiting bodies were transferred to microscope slides and mounted in acid fuchsin-lactophenol.

4. The Others:

Besides the methods mentioned above, there are some techniques occasionally used to induce the development of the perfect stage of *R. solani*. Sims (59) reports that basidiospores of *R. solani* occur on the surface of the autoclaved plant juice extracts of potato and alligator weed, to which are added 5 g./1 liter of sucrose.

Stretton *et al.* (57) mention that one isolate of *R. solani* on one occasion formed masses of basidia and spores over the surface of tap water in a Petri dish containing wheat and radish seedlings. The seedlings have been grown previously in sand inoculated with this isolate and are placed in water for examination of root lesions.

Factors Influencing the Formation of the Perfect Stage of R. solani:

The factors influencing the development of the perfect stage of *R. solani* are still not clearly known. The numerous papers dealing with the formation of the perfect stage of *R. solani* show that there appears to be marked differences in conditions required for fruiting among the different species of *Rhizoctonia* and among different isolate of *R. solani*. Because of this fact, to clarify these factors which influence the formation of the perfect stage of all isolates of *R. solani* is very difficult. However, by the literatures at hand, the factors can be summerized as following:

1. Substrate:

As mentioned in the above section, there are commonly 3 kinds of substrates used for inducing *R. solani* to fructify. They are soil, agar, and plant tissues. Different isolate of *R. solani* requires different substrate for fruiting. Stretton *et al.* (60) point out that all isolates of *Thanatephorus praticolus* fruit on agar but not on the soil surface; pathogenic isolates of *T. cucumeris* fruit on soil but not on agar; non-pathogenic isolates of *T. cucumeris* fruit both on agar and soil.

It is not true that all kinds of soil can be used to induce *R. solani* to fruit. Stretton *et al.* (60) mention that pathogenic isolates of *R. solani* (=*T. cucumeris*) differ in their ability to fruit on different soils. Flentje (20) reports that the Waite Institute soil (a heavy red-brown silt loam, wilting point, 6-8% moisture; field capacity, 20-22% moisture; PH 6.5) is the pest one for fructificaton. He also points out that *R. solani* will fail to fruit when Waite Institute soil is added Krillium at the rate of 1:300 or autoclaved. Stretton *et al.* (60) report that coarse river sand, red-brown loam, and two different clay loams have been tested with reasonally uniform success.

When agar is used as a substrate to induce fructification, the concentration of agar is an important factor. Hawn and Vanterpool (26) report that 1% of water agar is not as satisfactory as 2%. Some isolates only fruit on soil-extract agar (39).

Plant tissues of cotton, alligator weed, wandering jew, potato and sugar beet are commonly used as substrates for inducing fructification (18, 59). Flentje (20) states that fructification develops equally well with or without plants in the soil.

2. Light:

Light plays an important role in the development of fructification. Carpenter (7) reports that the heaviest spore production occurs at night. Sporulation is sharply reduced even by diffuse day light. Stretton *et al.* (60) state that fructifications occur at 8-24 hr. light periods of constant or fluctuating light intensities of 4-1,450 ft-c. Flentje (20) mentions that the hymenium is formed under light intensities varying from 200 to 300 up to 2,000 ft-c. The fact that a wide range of the light intensity can equally induce the fructification shows that the light period is probably more important than light intensity.

3. Humidity

The requirement of humidity for fructification varies from isolate to isolate. Stretton *et al.* (60) state that the optimum relative humidity appears at 40-60%. Sims (59) and Kotila (30) report that the fructification occurs at a relative humidity of nearly 100%.

High relative humidity is more favorable for the formation of the perfect atage. In the field, Carpenter (7) reports that the heavy sporulation occurs at increased humidity, commonly accompanied by a heavy dew formation that moistens the leaves of the host and provides conditions favorable for both the production and germination of basidiospores.

When the soil is used as a substrate to induce *R. solani* to fructify, the humidity of the soil surface is assumed to be much higher than the corresponding atmospheric humidity (62).

4. Temperature:

Temperature does not apear to be critical. Flentje (20) reports that fructification develops under green house conditions in both summer (average maximum temperature 95 F, average minimum temperature 65 F) and winter (average maximum temperature 65 F, average minimum temperature 48 F). Commonly, the temperature required for the fomation of the fructification varies from 20-30 C (62). According to Sims (59), Kotila (30), Flentje (20), Kotila (31), Ullstrup (62) respectively, the optimum temperature is 20 , 21-25 , 25 21 and 27-29 C.

5. Inoculum:

The inoculum is also a very important factor. Flentje (20) states that fructifications are slightly reduced when the rate of maize meal-sand inoculum is increased from 2.5 to 5%, and are further reduced if the soil is inoculated only with a suspension of mycelium in distilled water. Sims (59) reports an opposite opinion and says that fructifications occur only when mycelial suspension is used. No fructifications occur when inoculum is mycelial mates.

The different results reported by these two investigators are apparently due to the methods used for inducing the formation of the fructification. The former uses soil-plate method; the latter uses plant tissue method.

The age of inoculum also has a great influence on the fructification when the water agar method is used to induce the formation of the perfect stage of *R. solani*. Hawn and Vanterpool(26)report that inocula from 4-, 7-, and 14-day-old cultures give only mycelial and sclerotia growth. The 30-day-old culture inoculum is the best one.

Discussion and Conclusion

R. solani (=*Thanatephorus cucumeris*) was first described by Kühn in 1858 as a pathogen on potato. Its perfect stage was not connected together until Rolfs (48)

proved that the perfect stage was *Corticium*. For a long time, the perfect stage had been referred to either *C. vagum* var. *solani* Burt or *C. solani* (Prill. and Dell.) Bourd. and Calz. After 1943, the perfect stage of *R. solani* was regarded as *Pellicularia filamentosa* (Pat.) Rogers.

Donk (10, 11, 12, 13) with a series of discussion on this fungus, considered that *P. filamentosa* was a *nomen confusum*, and recombined the fungus with its various synonyms in the new combination *Thanatephorus cucumeris* (Frank) Donk.

Warcup and Talbot (65) supported the opinion of Donk, and regarded the name *T. cucumeris* was the correct name of the perfect stage of *R. solani*.

The asexual structures of *R. solani* are not specific. mycelium of many species of *Rhizoctonia* resemble the mycelium of *R. solani* (42). Even within the species of *R. solani*, the asexual structures vary among strains. *R. solani* is considered a complex species (26). Parmeter *et al*, (42) point out that *Ceratobasidium* sp. and other *R. solani*-like fungi have often been mistaken for *R. solani*. The only difference between *T. cucumeris* and *Ceratobasidium* sp. is the number of nuclei in the sterile hyphal cells. The former is multinucleate, and the latter is binucleate.

The confusion of the asexual structures between *R. solani* and its similar fungi creates a serious problem. In the absence of the observations in nuclear condition and perfect stage, the results cf the past data on variation in *R. solani* is questionable in many cases (42). Thus, for clarifying this confusion and the future studies of this fungus, to develop a simple method for inducing the production of the perfect stage of *R. solani* and its similar fungi *in vitro* becomes more and more important today.

In early years, many investigators (2, 58) reported that it was found impossible to get the perfect stage of *R. solani* in cultures on artificial media. In 1924, Muller (29) first induced the production of the perfect stage of *R. solani* in the laboratory by water agar method. Since that time, many techniques have been developed. Among them, 1)fructification on agar, 2) fructification on plant tissue, and 3) fructification on the soil surface are the most practical and commonly used.

These three techniques can not induce all strains of *R. solani* to produce the perfect stage. Stretton *et al*. (60) report that all isolates of *T. praticolus* fruit on agar but not on soil surface; pathogenic isolaes of *T. cucumeris* fruit on soil surface but not on agar; nonpathogenic isolates of *T. cucumeris* fruit both on agar and soil. The reason is not known.

Facfcrs influencing the formation of the perfect stage of *R. solani* are not completely known today. Vitamins, growth-promoting substances, plant extracts, and several natural and synthetic compounds are reported no influence on the formation of the perfect stage (39). Some factors, such as substrates (kinds of soil, plant tissue, and perulation agar), light, moisture, temperature, and inoculum are known to have more or less influence on the development of the perfect stage of *R. solani*.

Since the efficient technique is still lacking, and the factors influencing the development of the perfect stage are not completely known, the observation of nuclear condition in the sterile hyphal cells is the best and easiest way to distinguish *R. solani* from its similar fungi.

Further detailed studies on the factors influencing the development of the perfect stage of *R. solani* are necessary. The understanding of these factors may lead to develop a suitable technique for inducing the development of the perfect stage on artificial media under controlled conditions.

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Rhizoctonia solani Kühn 之形態學及誘導產生有性世代之方法

杜　金　池

中 文 摘 要

Rhizoctonia solani 於 1858 年首被 Kühn 氏發現，其有性世代亦早於 1891 年即被描述，惟直到 1903 年 Rolfs 氏證明其有性世代爲 Corticium sp. 以前，未見有連結其有性世代爲同一種眞菌之不同世代的報告。自玆而後，*R. solani* 之有性世代即被描述爲 *C. vagum* var. *solani* Burt 或 *R. solani* (Prill. and Del.) Bourd and Calz。1943 年後，本菌之有性世代被 Rogers 氏更名爲 *Pellicularia filamentosa* (Pat.) Rogers.

本病原菌被更名爲 *P. filamentosa* 曾引起國際間眞菌學者之爭論。Donk 氏經一連串之討論，並提出強烈證據，認爲 *P. filamentosa* 是 *nomen confusum* 違背國際植物命名法，是一種不合法之定名。Donk 氏經重新整理並綜合異名同種，認爲本病原菌之有性世代應爲 *Thanatephorus cucumeris* (Frank) Donk.

Donk 氏之意見受到多數眞菌學者之同意。Warcup 及 Talbot 兩氏曾強烈支持 Donk 氏之意見，並認爲 *T. cucumeris* 是 *R. solani* 最正確之有性世代學名。

R. solani 無性世代之菌體並無特殊性。多數 *Rhizoctonia* 屬內之種菌絲性狀並無分別，且同種內之不同系統無性世代之菌體變化極大，是以 *R. solani* 被認爲是混合種。Parmeter 等氏曾指出 *Ceratobasidium* sp. 及其他 *R. solani* 之類似菌曾長期被誤認爲 *R. solani*。*T. cucumeris* 與 *Ceratobasidium* sp. 之無性世代菌絲體，惟一不同處僅爲菌絲細胞內細胞核之數目。前者具多數細胞核；後者則僅含有兩個細胞核。

R. solani 及其類似菌間之混亂，引起眞菌學及植物病理學上嚴重紛亂。過去在缺少細胞核情形及有性世代之觀察下，關於 *R. solani* 之報告實値得懷疑並重新檢討。因此爲澄清日後之混淆，發展一種可在研究室進行之簡單而容易的誘致 *R. solani* 及其類似菌類產生有性世代之方法至屬必要。

早年依據諸多學者報告，本病原菌不可能在人工培養基上產生有性世代，1924年在實驗室 Muller 氏首先以水瓊脂法培養本菌而得到有性世代，自此而後很多方法即被研究並發展之，但仍未得到最理想方法。在這些已被知道的方法中，約可分成三類：1）瓊脂法，2）植物組織法，3）土壤法。

上述三種方法並不能誘致 *R. solani* 之所有系統及其類似菌類產生有性世代。蓋 *Rhizoctonia* 產生有性世代之條件，因系統不同而異，並不能以一概全。Stretton 等氏曾報告，在其試驗使用之 *T. praticolus* 各菌株只可在瓊脂上產生有性世代；具植物病原性之 *T. cucumeris* 只能在土壤之表面產生有性世代：無植物病原性之 *T. cucumeris* 則在瓊脂上或土壤表面上均可產生有性世代。何以如此，原因不明。

迄至目前，控制 *Rhizoctonia* 產生有性世代之因子尙未充分瞭解。據 Papavizas 氏之報告，維他命、生長促進物質、植物抽出液、及許多天然或人工化合物均與本菌產生有性世代無關。某些因子如培養基質、光線、濕度、溫度和接種源則或多或少可影響本菌產生有性世代。

綜上所述，在有把握而可供 *R. solani* 及其類似菌產生有性世代之方法尙未發現，影響產生有性世代之因子亦未充分明瞭之前，菌絲體內細胞核數目之觀察爲初步區別 *R. solani* 及其類似菌之最簡便方法。筆者並非主張眞菌之鑑定，僅可依據細胞核之數目，而是在目前情形下，如以上述各種誘致產生有性世代之方法，誘導 *R. solani* 及其類似菌產生有性世代尙不可得時，惟有註明菌絲體內細胞核情形。比未註明菌絲細胞核情形就列爲 *R. solani* 更清晰而正確。

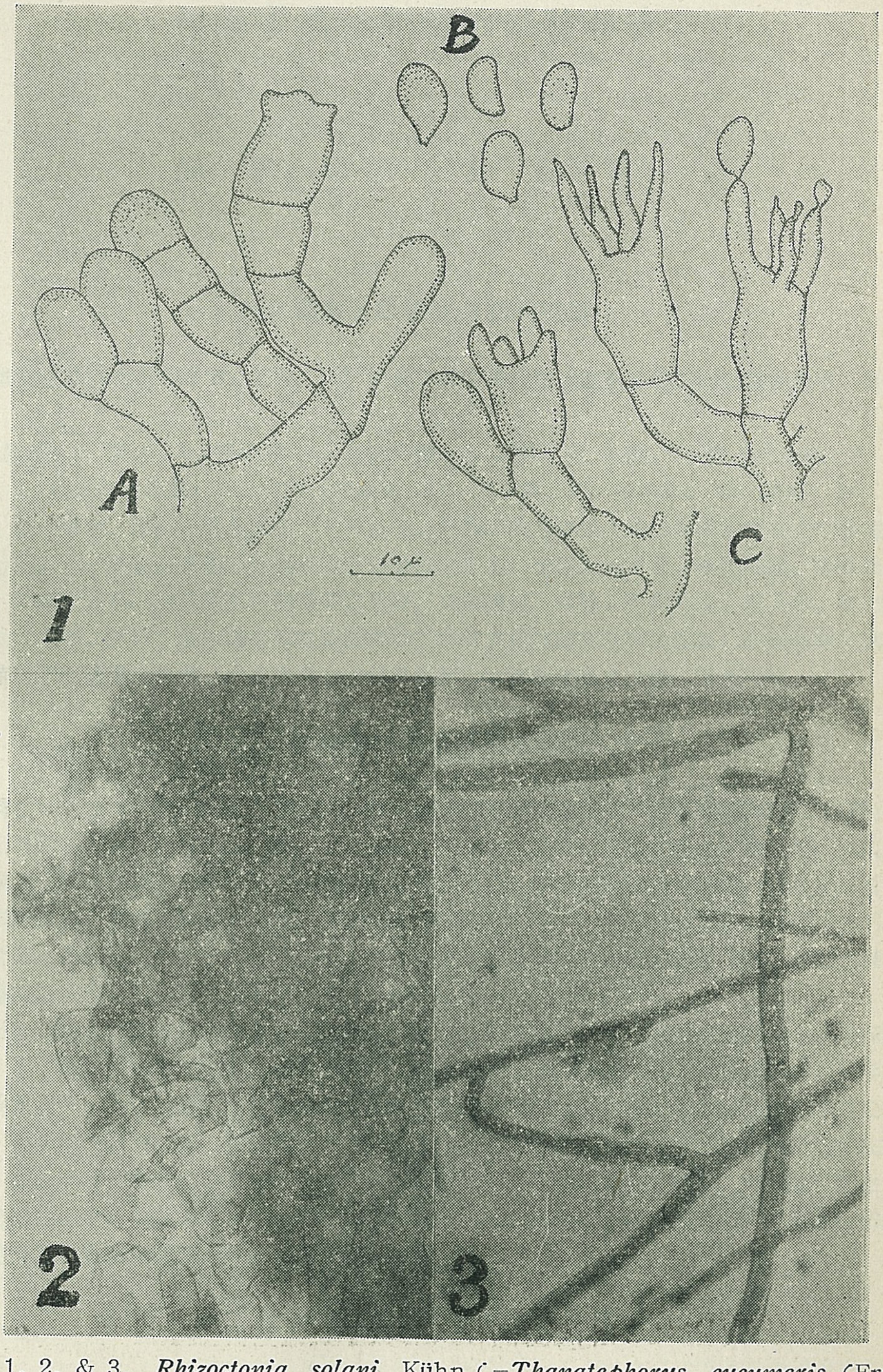

Figure 1, 2, & 3. ***Rhizoctonia solani*** Kühn (=***Thanatephorus cucumeris*** (Frank) Donk.) 1. A. Hymenial branches. B. Basidiospores. C. Basidia. 2. Sclerotial structure (x 450). 3. Hyphae and its branches (x 450).